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PRIMER NOTE

Sixteen microsatellite loci from *Halesia tetraptera* (Styracaceae)

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Abstract

Sixteen microsatellite loci isolated from *Halesia tetraptera* are described. All 16 are polymorphic, with four to 13 alleles across 15–19 members of a single half-sib family. Heterozygosity ranged from 0.067 to 1. One locus departs significantly from Hardy–Weinberg equilibrium in our test family. The test population shows significant heterozygote deficiency at this and two other loci. Thirteen loci exhibit significant linkage disequilibrium. These loci will be utilized in paternity analyses of geographically diverse half-sib families.

Keywords: Carolina silverbell, *Halesia*, microsatellite, population genetics, SSR

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Halesia tetraptera J. Ellis (Styracaceae), Carolina silverbell, is a small tree or a large shrub found in the understory of Appalachian deciduous forests throughout the southeastern USA. Although Fritsch & Lucas (2000) argued that the species is not distinct from *Halesia carolina* L., a Coastal Plain species, we are following the USDA-ARS Germplasm Resources Information Network in recognizing the two as separate entities (http://www.ars-grin.gov/npgs).

The US National Arboretum sponsored extensive germplasm collections of *H. tetraptera* from 1992 to 1994 in the form of seed lots from selected trees in populations from seven states. The collections represent half-sib families from diverse samples throughout the broad range of the species. We felt it would be valuable to develop a microsatellite DNA library for the species for various diversity and population studies. In this paper, we report on the first 16 loci that we have successfully isolated and tested from the Carolina silverbell.

We isolated microsatellite loci from *H. tetraptera* using a modification of the enrichment method of simple sequence repeat (SSR) marker development of Edwards *et al*. (1996). Genomic DNA was restricted, ligated to adaptors and amplified with polymerase chain reaction (PCR). The amplicons were hybridized twice with biotin-labelled synthetic SSRs and isolated using streptavidin coated beads (Dynal) in conjunction with a Dynal magnetic particle concentrator. The eluted fragments were size separated using Sepharose CL-4B SizeSep 400 Spun Columns (Amersham Pharmacia Biotech), amplified and cloned (using phage and plasmid vectors with M13 priming sites), and the clones were screened by sequencing. Approximately 70% of our clones contained a repeat. Reverse sequences were obtained for these, and primers were designed from the flanking regions. The primers were first tested using labelled dNTPs on the genomic DNA from which they were isolated and a few individuals from other populations. If successful and polymorphic, a fluorescently labelled forward primer was subsequently obtained. The 16 primer pairs (Table 1) were tested across a half-sib family from Rutherford County, North Carolina. Differences in allele size were detected on an ABI 3730 Genetic Analyser (Applied Biosystems) using capillary gel electrophoresis with GeneScan ROX-500 size standard (Applied Biosystems). PCR mix for all primers was 1.0 μL betaine, 1.0 μL 10× buffer with 15 mM $MgCl_2$, 0.2 μL 10 mM dNTPs, 0.25 μL each forward and reverse primer, 0.05 μL *Taq* DNA polymerase (New England Biolabs), 1.0 μL genomic DNA template and 6.25 μL dH_2O for a total volume of 10 μL. We used a touchdown PCR program for all primers: 5 min at 94 °C, 14 cycles of [45 s at 94 °C, 45 s at 60 °C (dropping 1 °C each cycle), 1 min at 72 °C], 25 cycles of (45 s at 94 °C, 45 s at 46 °C, 1 min 72 C°), 10 min at 72 °C and 4 °C storage. Preliminary analysis of raw microsatellite data was performed using

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Table 1 Primer sequences and characteristics of 16 *Halesia tetrapetra* microsatellite loci

Locus	GenBank Accession no.	Forward primer (5′–3′)	Reverse primer (5′–3′)	Repeat	T_m (°C)	*n*	No. of alleles	Allele size range (bp)	Mean H_E	Mean H_O	*f*
Ha1	AY947643	AGTGCCTTCTCAAGTTTACTG†	GGTGCCTTTGAAAGGGTAATG	$(AG)_{15}$	60	19	9	122–147	0.821	0.789	0.039
Ha2	AY947644	CCTGTAATATGAGAGCGAAGTG†	TGTGGGCATTCTGGCATTC	$(GAG)_{10}$	60	19	6	109–127	0.757	0.737	0.027
Ha3	AY947645	CTCAGAAAGCTCGACGAAC‡	TCAGTGAAAATGATGTGGCAG	$(CT)_4(CC)(CT)_{28}$	60	19	11	201–247	0.890	0.895	–0.005
Ha4	AY947646	CCTAGTGTAGTGGCCCTTTC§	ACAAAGAGCATCCAATCAACAG	$(GA)_{23}$	61	17	9	346–390	0.761	0.765	–0.005
Ha6	AY947647	TGAAAATTCGCCAACGAGG†	GTTAGTCGCTATCAACTCCAG	$(GA)_{11}$	59	19	4	111–121	0.563	0.632	–0.125
Ha7	AY947648	TTGGAGATGGTGGAGGAAG†	TGCACTGTTAAGTTCTGAAGTC	$(AG)_{22}$	60	19	10	124–164	0.866	0.895	–0.034
Ha11	AY947649	GGTGTTTGCAGGACAAAATTG‡	GTGGGAAGAATGAAAGGTATGG	$(CTT)_{12}$	60	19	7	185–228	0.752	0.789	–0.051
Ha12	AY947650	ATACTACCCATTTGCATTGGAC†	GCACTTGAACCGCTTGAAC	$(GA)_{13}$	59	19	10	112–145	0.858	0.789	0.082
Ha13	AY947651	CCATTTCCAACTCACAAAACC†	CATGTTTCACCTCCTTGACTC	$(GA)_{18}$	60	19	9	109–133	0.839	1.000	–0.198
Ha15	AY947652	CATCAAACACCCCAAGTTCAG‡	CGAGGAGGGAAGTTATTGTCAG	$(AG)_{11}$	62	19	4	209–215	0.698	0.579	0.175
Ha18	AY947653	GCAACTCCTTTCACGTAGTC‡	CACAAGCACCTCTCTGTAATC	$(AG)_{13}$	60	19	7	217–233	0.711	0.842	–0.190
Ha19	AY947654	CGCAATTCTTCTTTGACGAC†	TCAAGCACAACAATAAGGGAC	$(CT)_{19}$	58	15	8	139–172	0.811	0.067*	0.920
Ha21	AY947655	ACACCCCCCAATTAACACC§	AGACATTCTCTCTGCTGCC	$(AG)_{35}$	60	17	13	329–423	0.882	0.765	0.137
Ha22	AY947656	GGACAAGAGAGAGAGAAAGGG§	GAGCACTATTATGTGGCAAGG	$(AG)^5(AAAGGG)(AG)_{12}$	62	15	4	276–294	0.729	0.400	0.460
Ha23	AY947657	TGGGTAATAGGTAGAGGAGGAG§	TGTCATCGGTAAGTAGCAGTAG	$(TTC)_{10}$	62	18	7	334–355	0.759	0.944	–0.254
Ha24	AY947658	ATAGAATTAAGTGCAGCAGAGG‡	CATTGGGATGTGGTGATGAG	$(GA)_{18}$	59	17	10	177–222	0.868	0.941	–0.088

n, sample size; T_m, annealing temperature; H_E, expected heterozygosity; H_O, observed heterozygosity; *f*, estimate of fixation index.
†Labelled with 6FAM fluorescent dye.
‡Labelled with HEX fluorescent dye.
§Labelled with NED fluorescent dye.
*Departs significantly from HWE at $P < 0.05$.

GENEMAPPER 3.5 (Applied Biosystems). Descriptive statistics (Table 1) were generated with GDA version 1.1 (Lewis & Zaykin 2002). Tests for Hardy–Weinberg equilibrium (HWE, exact test) and linkage disequilibrium (LD) were run with GENEPOP 3.4 (Raymond & Rousset 1995).

None of the loci is monomorphic across the test population. One locus (Ha19) departs significantly from HWE ($P < 0.001$) in our test half-sib family. The test family shows significant heterozygote deficiency at this locus, and also at Ha21 ($P < 0.05$) and Ha22 ($P < 0.005$). Whether this indicates the presence of null alleles at these loci has not yet been determined. Heterozygote excess is only apparent at two loci, Ha13 and Ha23 ($P < 0.05$). Thirteen of the loci showed significant LD ($P < 0.05$) with at least one other locus, which is likely not due to physical linkage in all cases, but the fact that our test population is a half-sib family (Flint-Garcia *et al*. 2003). We have not tested our primers with DNA from other *Halesia* species.

We plan to use these SSR loci to examine geographical variance in paternity among half-sib families of *H. tetraptera*. We are hoping to recover sufficient additional microsatellite loci from our insert library in order to perform association mapping with phenotypic data collected from the same progeny.

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